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Impact of exogenous applications of salicylic acid on growth and physiochemical attributes of *Trigonella foenum-graecum* L. grown under sodium fluoride stress

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ABSTRACT

Purpose: A major issue around the world, particularly in arid regions, is the salinization of arable land, which has a deleterious impact on plants and poses a threat to the world's food supply. Fluoride is an extremely harmful contamination in soil that destroys crops on a large scale. Nevertheless, *Trigonella foenum-graecum* L. (fenugreek) growth is continuously declining due to the salinity issue. Fenugreek has a wide range of pharmaceutical significance and contains many essential minerals.

Methods: Seed priming with salicylic acid is therefore a remarkably efficient way to increase fenugreek production in salinity-affected soils. The effects of sodium fluoride (200 ppm NaF) on fenugreek were investigated in a pot experiment. The fenugreek seeds were primed with 5, 10, and 15 µmol L^{-1} of salicylic acid.

Results: NaF was found to substantially reduce the length of the shoot (17.3 %) and root (39.5 %) in addition to reducing the area of the leaf (31.9 %) and root nodules (24.7 %), when compared to the control seedlings. NaF also caused reductions in photosynthetic pigments, stomatal conductance (40 %), transpiration rate (41.1 %), and photosynthetic rate (32.2 %). Salicylic acid primed seed reduced the salinity stress by improved growth along with enhanced production of proline (94.5 %).

Conclusions: The treatment with SA2 (10 mol L^{-1}) had the most significant effect on fenugreek biomass, total chlorophyll content (62.3 %), and gas exchange parameters in control as well as NaF-stressed seedlings suggesting that exogenous SA could be employed to improve plant tolerance to NaF stress.

Key-words: Fenugreek, growth, proline, salicylic acid, sodium fluoride.

iNTRODUCTION

Plants endure several kinds of abiotic stresses like salinity, heavy metals, drought, extreme temperatures, and nutrient deficiencies because of their sessile nature. Due to these constraints, the production of crops is hampered and the global supply of food is disrupted[1]. Man-made practices in the developmental age have exacerbated agronomics deterioration due to biotic pressures[2]. The world's population is expanding day by day, which exerts pressure on the food supplies, so it becomes inevitable to prevent such kinds of losses.[3] Biosynthesis of essential compounds as well as the availability of nutrients is altered because of these environmental constraints.[4]

The main abiotic constraint that is impeding normal plant growth and development all around the world is salinity.[5] Salt stress is a critical environmental agent that impacts crop yield and productivity, particularly in arid and semi-arid regions.[6] Depending on the duration or intensity of the stress, plant's numerous physiological and biochemical activities are impaired, reducing crop production.[7] Salinity has negative impact on plant development, photosynthesis, germination, photosynthetic pigments (chlorophyll *a*, *b*, and carotenoids), nutrient imbalance, water retention, and yield.[8] Plants confront a problem as a result of salt. Inorganic mineral supplementation interrupts osmotic potential and induces osmotic stress in plants.[9]

Fluoride is a highly reactive, non-biodegradable, and ubiquitous pollutant present in the environment.[10] Fluoride deposition can occur from manmade or anthropogenic sources.[11] Toxicity of fluoride results in a reduction in shoot and root length, percentage germination of seed, and biomass production in comparison to control. Fluoride toxicity causes significant reductions in radical and plumule lengths.[12] It shows negative impact on nutrient uptake, the metabolic activity of plants, physiological processes, enzymes as well as protein synthesis, germination of seeds, growth, and productivity of plants.[13] Sodium fluoride has a significant relationship with plants and negatively influences several biochemical and physiological traits without necessarily manifesting any obvious signs of harm. By interfering with several metabolic processes, including respiration and photosynthesis, NaF influences the plant's various developmental processes.[14]

Farmers' ultimate focus nowadays is to boost their crops' resilience to such stressors, which can be accomplished by selecting the appropriate strategies.[15] Horticulture crops and field crops are both commercially significant, so they must be resistant from abiotic stresses. They require more resources for development yet are essential for a balanced diet's supply of fibers, nutrients, and minerals.[16] To increase resistance to stress, several strategies can be utilized; some require a long time, such as traditional breeding methods, while others, such as gene modification, are banned in many countries throughout the world. The desired yield in the future cannot be attained with present agricultural technologies or by growing tolerant crop varieties.[17] Instead of these tactics, seed priming allows plants to better survive abiotic environments.[18] Seed priming is the most promising strategy for increasing plant growth rate.[19] Seed priming with high antioxidant concentrations is an effective technique to reduce fluoride toxicity in agricultural systems.[10] Before the process of germination, a primed state promotes multiple physiological responses.[20] This is accomplished through seed priming with several phytohormones or antioxidants. Even under severe or salinized environments, primed seeds germinate better and cause more synchronized germination.[21] It contributes to many biochemical, molecular, and physiological changes in plants, as well as improving nutrient uptake, hormone production, and the antioxidant system.[22]

Salicylic acid antioxidant is one of the phytoprotectants that should be used to improve growth and increase the production or yield of species that are affected by abiotic stressors. It is critical for protecting plants from abiotic environments.[23] Salicylic acid is essential for stomatal development, flowering, nodulation, and closure. It has a promising role in cell proliferation as well as seedling germination.[24] SA modulates critical activities like ion transport and nutrition uptake under salt stress.[25] Salicylic acid is applied exogenously to plants under salt stress, either by soaking seeds or by spraying or irrigating with a nutritional solution. This activates a salt tolerance mechanism in response to various abiotic conditions.[26] SA reduces reactive oxygen species (ROS) by strengthening the antioxidant defense system and increasing plant membrane integrity.[24] Salicylic acid treatment increases abscisic acid content, resulting in the production of anti-stress proteins that promote plant health under stress.[27]

Trigonella foenum-graecum L., (fenugreek), belongs to the fabaceae, or leguminosae, family. This family of plants is a significant source of oils, fuel, wood, dyes, gums, pesticides, pulses, and medicines.[28] Since ancient times, fenugreek has been utilized all across the world as a medicinal plant. Fenugreek develops very rapidly. It can grow in plains, fields, dry grasslands, hillsides, cultivated lands, and uncultivated grounds.[29] Fenugreek has been used as a medicinal plant for centuries because of its hypocholesterolemic and anti-diabetic properties. As a spice and smelling agent, it is also employed. Its seeds contain 0.2%–0.9% diosgenin, 4.8% saponins, 10% flavonoids, and 35% alkaloids. Fenugreek is an annual, dicotyledonous plant.[30] Fenugreek is less productive and yielding than other legumes due to abiotic stress. So it is suggested to improve phytochemical qualities and productivity of fenugreek with the aid of various agricultural systems or technologies can be attained.[31] Therefore, the current study was designed to investigate the influence of salicylic acid-priming on seed germination, physiochemical attributes and growth of fenugreek under NaF stress.

MATERIAL AND METHODS

Experimental design and treatments

Trigonella foenum-graecum L. (fenugreek, variety Kasuri) seeds were acquired from a local market in Lahore. Fenugreek seeds were surface sterilized by soaking them in sodium hypochlorite solution (NaOCl, 0.5%) for 3 minutes. Fenugreek seeds were primed in various concentrations of salicylic acid (SA) solutions (5, 10, 15 µmol L^{-1} designated as SA1, SA2, and SA3 respectively) for 24 hours at room temperature. Salicylic acid primed seeds were washed thoroughly in distilled water and air dried at room temperature. The control treatment consisted of non-primed fenugreek seeds. The experimental site was selected in the Botanical Garden of the University of the Punjab, Quaid-e-Azam Campus, Lahore. The nine seeds per pot were sown in the arranged 24 pots on November 18, 2021. There was a total of 24 pots, which were divided into eight groups. Each group had 3 replicates with a randomized complete block design (RCBD). After 30 days of germination, 10ml of 200ppm solution of NaF solution was given to plants labeled as NaF and the method of application was soil drench. A total of four NaF treatments were given, and the interval between two successive NaF treatments was 2 days. After completion of the field experiment, a harvest was taken (40 days after germination) to examine the physio-biochemical characteristics and growth of fenugreek.

Assessment of morphological and biomass attributes

Harvest was taken after 40 days of germination and plants were selected randomly from each of the treatments. Root length, shoot length, branches number, total number of leaves, leaf area, number of root nodules, fresh along with the dry weight of shoots as well as roots were analyzed. Leaf area was calculated by using the formula of Charleton and Foote (1965). Fresh and dry biomass was measured by electrical balance (Sartorius GMBH, type 1216MP 6E, Gottingen, Germany). For the assessment of dry weight, fenugreek plants were first dried in air and then kept in the drying oven (Wiseven, Model WOF-105, Korea) for 48 hr.

Measurement of leaf gas exchange parameters

Transpiration rate (*E*), stomatal conductance (*gs*), and rate of photosynthesis (*A*) were evaluated by availing IRGA (Infrared Gas Analyzer) LCA-4 system (ADC. Ltd) device at 9:30 to 10:30 am.

Determination of Photosynthetic Pigments

Plant photosynthetic pigments such as chlorophyll *a*, chlorophyll *b*, and total chlorophyll were calculated by using the method of Arnon (1949) while carotenoid content was measured through the Davis method (1976). Fresh fenugreek leaves were crushed by using a pestle and mortar and extracted with 80% acetone solution in concentration of 10ml. This was then stored at low temperature. Centrifugation of extract was done at the temperature of 4ºC, at speed of 10000 rpm for approximately 5 minutes. The Optical density of the obtained supernatant was determined at 663nm, 645nm, and 480nm on the spectrophotometer (Shimadzu UV-1800).

Estimation of Proline content

Plant leaves (0.25g, ice chilled) were taken for measuring Proline content. These were then vortexes after the addition of 10ml Sulpho-salicylic acid (3%). Then filtration was done with the help of Whatman's no. 2 filter paper. Along with 2ml Ninhydrin (72.8ml acetic acid, Ninhydrin (2.8g), 85% phosphoric acid (48.16ml), 2ml glacial acetic acid in addition to 2ml of the filtrate were taken. The mixture was homogenized by keeping on a water bath (N.S Engineering Concern, Model XMTG-9000) at 100ºC. After that, the mixture was cooled for one hour. It was then vortexes for 30 seconds after the addition of 4ml toluene. The chromospheres, having toluene in it was used for aspiration. The solution was kept for 0.5 hours at 25ºC. At 520nm absorbance was taken on the spectrophotometer (Shimadzu UV-1800) and these values were compared with the standard curve of the Proline.

Statistical analysis

During the germination period of the fenugreek crop, the data obtained from the pot experiment was taken to measure standard error as well as mean error, and then this data was compared by ANOVA. This was done through software (IBM-SPSS Statistics version 20) by the application of Duncan's Multiple Range Test (DMRT) at p≤0.05 significant level. Pearson's Correlation Analysis was utilized to measure correlations between the numerous studied parameters. The Pearson correlation coefficients and Principal Component Analysis (PCA) between the fenugreek measured variables was assessed using R studio.

RESULTS

Regulation of vegetative and biomass parameters by SA priming: Sodium fluoride (NaF) toxicity caused a significant reduction in the morphological parameters of fenugreek seedlings. Plants treated with 200 ppm NaF had the greatest reduction in shoot length (17.3%) and root length (39.5%) of fenugreek seedlings. Shoot length was enhanced by 22.7% in SA2 plants as compared to control treatments. Salinity stress reduced the number of branches per plant, the number of leaves, the area of the leaves, and the number of root nodules (Table 1). The total fresh weight of NaF-affected plants showed a decline in biomass traits by 29.5% compared to the control. Under NaF stress, there was a decrease of 28.8% and 35.0% in the fresh weight of the shoot and root, respectively in comparison with the fresh weight of the control. Sodium fluoride affected plants, treated with salicylic acid priming, manifested better percentage growth or biomass as compared to those saline plants in which no antioxidant was applied (Table 2).

Determination of gas exchange parameters: The NaF toxicity reduced the stomatal conductance in fenugreek seedlings compared to control seedlings by 40%. Nevertheless, salicylic acid treatments significantly increased (81%) stomatal conductance in SA2 compared to the control. The results showed that stomatal conductance enhanced 66.7, 90 and 76.7% in SA1+NaF, SA2+NaF and SA3+NaF, respectively in comparison with sole NaF seedlings. The Photosynthetic rate was reduced due to saline stress as it is observed in case of the NaF toxicity while increased when salicylic acid was applied. In comparison with the photosynthetic rate of control seedlings, the maximum decrease was observed in NaF treatment (32.2%) while the maximum increase was seen in SA2 treatments (93.9%). Transpiration rate demonstrated reduction in growth of the NaF plants and a decrease of 41.1% was manifested in comparison with the control. Transpiration rate was augmented in seedlings primed with SA1, SA2 and SA3 by 74.4%, 96.7%, and 88.9%, respectively compared to the control (Figure 1).

Improvement of photosynthetic pigments by SA treatments: Decreased chlorophyll content and carotenoids were observed in seedlings under NaF stress. Salicylic acid-primed fenugreek seeds enhanced photosynthetic pigments in seedlings. Seed primed with SA2 had higher concentrations of chlorophyll *a* by 7%, chlorophyll *b* by 28.6%, total chlorophyll by 32.3%, and carotenoids by % than SA1 treatments. Total chlorophyll was decreased by 27.95 under NaF stress. Total chlorophyll content was enhanced in SA2 (62.3%), SA3 (31.2%), and SA1 (10%) when their average values were compared with control seedlings (Figure 2). Due to fluoride toxicity, a 27.9% decrease in carotenoid was noticed in these plants. Carotenoids content increased by 37.2% in SA1, 43.2% in SA2, and 37.8% in SA3, when compared to the control. A maximum percentage increase was observed in SA2+NaF (47) when their values were compared with NaF-affected plants (Figure 2).

Changes in proline content by applying NaF and SA:
Changes in proline content by applying NaF and SA: Proline plays a very beneficial role in plants under various abiotic stresses. Maximum proline content was determined in SA2+NaF while reduced proline content was observed in control seedlings. In the case of sodium fluoride, proline content increased by 34.9 % compared to the control. Fenugreek primed seed with salicylic acid increased by 24.4, 94.5, and 46.7% was visualized in SA1, SA2, and SA3, respectively when it was compared with the proline content of the control. Other treatments such as SA1+NaF, SA2+NaF, and SA3+NaF showed an increase of 7.6%, 14%, and 14.3%, respectively, compared to SA1, SA2, and SA3 correspondingly (Figure 3).

Improvement of photosynthetic pigments by SA treatments: Decreased chlorophyll content and carotenoids were observed in seedlings under NaF stress. Salicylic acid-primed fenugreek seeds enhanced photosynthetic pigments in seedlings. Seed primed with SA2 had higher concentrations of chlorophyll *a* by 7%, chlorophyll *b* by 28.6%, total chlorophyll by 32.3%, and carotenoids by % than SA1 treatments. Total chlorophyll was decreased by 27.95 under NaF stress. Total chlorophyll content was enhanced in SA2 (62.3%), SA3 (31.2%), and SA1 (10%) when their average values were compared with control seedlings (Figure 2). Due to fluoride toxicity, a 27.9% decrease in carotenoid was noticed in these plants. Carotenoids content increased by 37.2% in SA1, 43.2% in SA2, and 37.8% in SA3, when compared to the control. A maximum percentage increase was observed in SA2+NaF (47) when their values were compared with NaF-affected plants (Figure 2).

Pearson Correlation: The Pearson correlation depicted a relationship between different parameters of *Trigonella foenum-graecum* L. treated with sodium fluoride and different concentrations of salicylic acid (Figure 4). Carotenoid content of fenugreek plants was found to be positively correlated with root and shoot length, total number of branches, number of leaves, area of the leaf, number of nodules, fresh and dry weight of root, fresh and dry weight of shoot, chlorophyll a, chlorophyll b, total chlorophyll, photosynthetic rate, transpiration rate, and stomatal conductivity, while negatively correlated with proline content.

Principal Component Analysis: Principal Component Analysis depicted the relationship of various growth traits of *Trigonella foenum-graecum* L. treated with salicylic acid under NaF stress. The entire set of data was split into two parts, with Dim1 accounting for 89.8% and Dim2 for 5.3%. The majority of the data demonstrated connections, with Dim1 displaying positive values. PC1 variables demonstrated positive correlation with root length, shoot fresh weight, root fresh weight, total fresh weight, root dry weight, number of branches, stomatal conductivity, transpiration rate, photosynthetic rate, chlorophyll *a*, carotenoids concentration, and proline content in fenugreek.

Table 1. Influence of Salicylic acid antioxidant on morphological traits of *T. foenum-graecum* under NaF stress

Treatments	Morphological traits					
	Shoot length (cm)	**Root length (cm)**	**No. of leaves**	**Area of leaves (cm^2)**	**No. of branches**	**No. of root nodules**
Control	7.700±0.058b	7.333±0.120b	40.333±0.882b	1.283±0.149b	11.00±0.577b	33.67±1.856 b
NaF	6.367±0.088a	4.433±0.033a	27.000±2.517a	0.873±0.018a	8.33±0.882a	25.33±0.882a
SA1	8.533±0.088e	8.333±0.145d	45.667±2.728b	1.845±0.139cd	13.67±0.333cde	47.67±0.667e
SA2	9.967±0.088e	12.100±0.11e	48.000±2.646b	2.518±0.091e	17.67±0.882e	53.00±1.155f
SA3	9.433±0.088d	8.500±0.115d	44.667±4.910b	2.253±0.112d	15.33±0.882f	49.67±0.882e
SA1+NaF	7.733±0.120b	7.767±0.088c	40.000±2.309b	1.290±0.068b	11.67±0.333bc	37.33±0.882c
SA2+NaF	8.433±0.067c	8.000±0.115c	42.667±2.848b	1.64±0.073bcd	14.67±0.667de	43.33±0.882d
SA3+NaF	7.867±0.088b	7.800±0.058c	40.667±2.333b	1.580±0.126b	12.67±0.333bcd	39.67±0.882c

Each treatment value is mean of 3replicates and ± values indicate standard errors. Significant difference between treatments is indicated by non-identical letters at p≤0.05.C=Control, NaF= 200ppm NaF, SA1= 5µM SA, SA2=10µM SA, SA3= 15µM SA

Table 2: Impact of salicylic acid on biomass assessment of *T. foenum-graecum* under NaF stress

Treatments	Biomass trait			
	Shoot fresh weight (g $plant^{-1}$)	**Root fresh weight (g $plant^{-1}$)**	**Shoot dry weight (g $plant^{-1}$)**	**Root dry weight (g $plant^{-1}$)**
Control	1.030±0.006b	0.123±0.003b	0.117±0.003b	0.023±0.0001b
NaF	0.733±0.067a	0.080±0.006a	0.073±0.009a	0.015±0.001a
SA1	1.213±0.043c	0.197±0.009d	0.150±0.006d	0.038±0.000c
SA2	1.577±0.009e	0.227±0.009e	0.217±0.009f	0.044±0.003d
SA3	1.420±0.012d	0.220±0.006e	0.180±0.006e	0.040±0.001c
SA1+NaF	1.033±0.054b	0.127±0.007b	0.123±0.009bc	0.024±0.001b
SA2+NaF	1.333±0.023d	0.153±0.007c	0.143±0.009cd	0.028±0.0029b
SA3+NaF	1.137±0.003bc	0.130±0.006d	0.130±0.006bcd	0.025±0.0005b

Each treatment value is mean of 3 replicates and ± values indicate standard errors. Significant difference between treatments is indicated by non-identical letters at p≤0.05.C=Control, NaF= 200ppm NaF, SA1= 5µM SA, SA2=10µM SA, SA3= 15µM SA

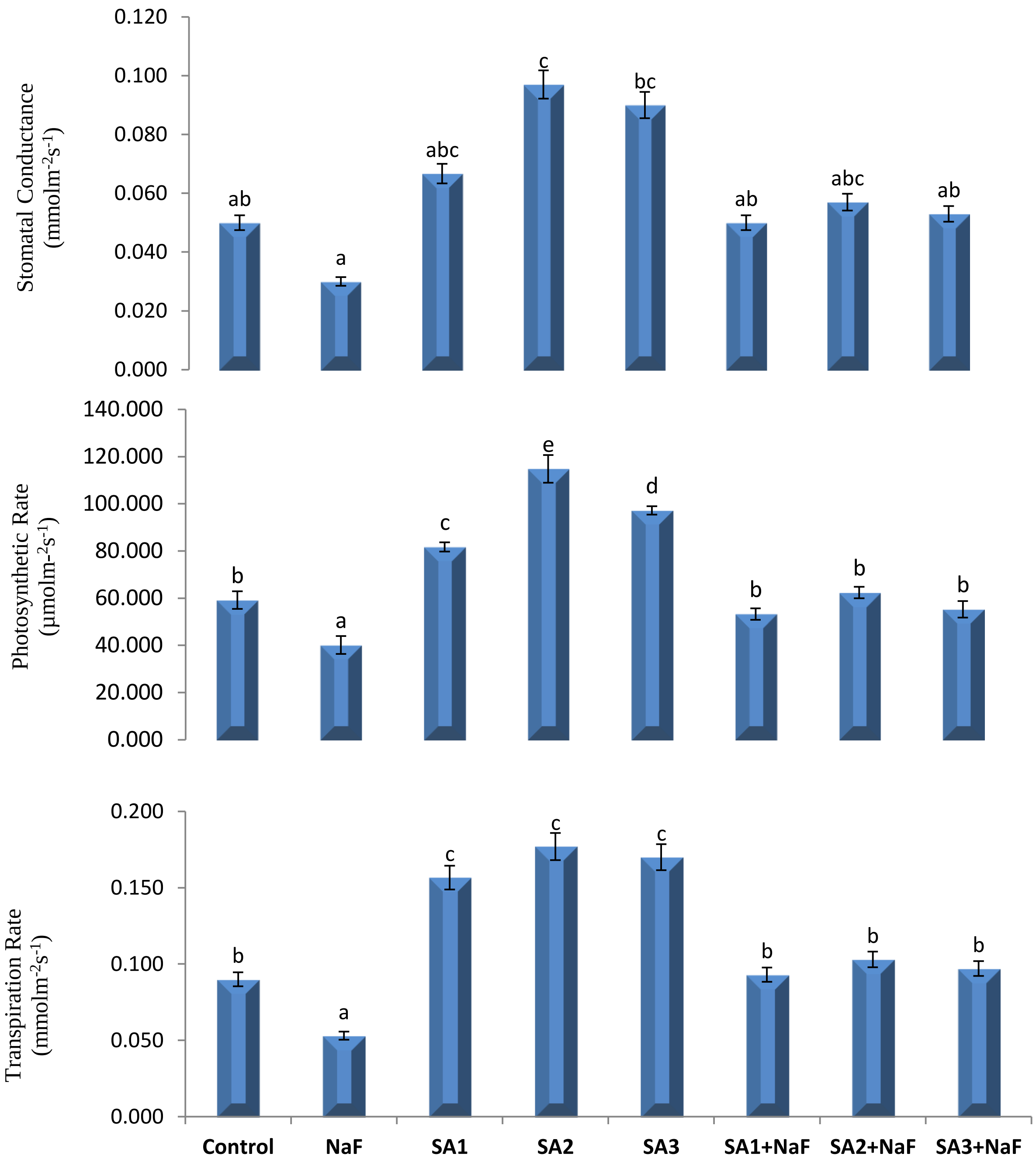


Figure 1: Impact of salicylic acid on leaf gas exchange parameters of fenugreek. Each value is mean of 3 replicates and ± values indicate standard errors. Significant difference between treatments is indicated by non-identical letters at p≤0.05. C=Control, NaF= 200ppm NaF, SA1= 5µM SA, SA2=10µM SA, SA3= 15µM SA

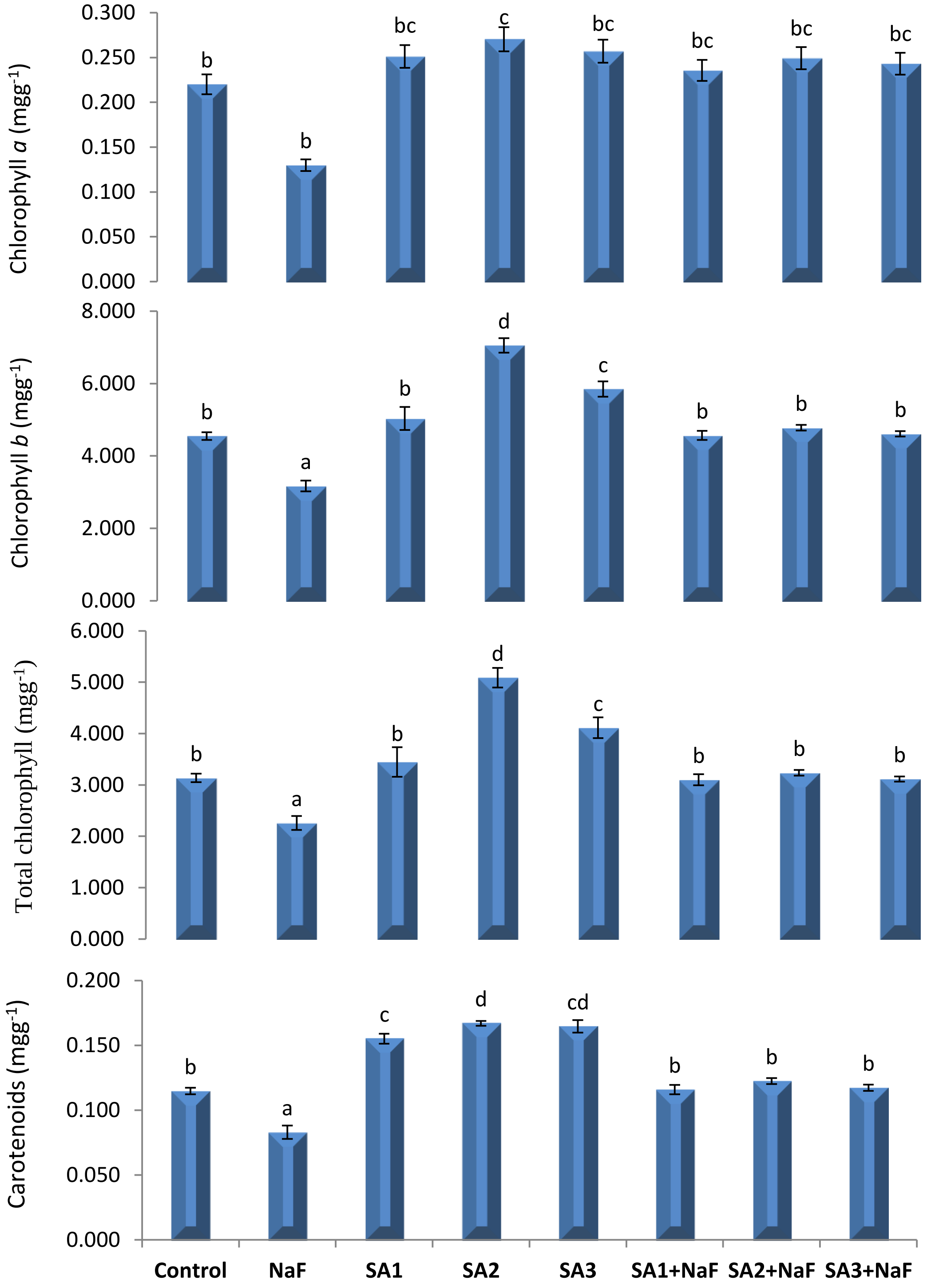


Figure 2: Effects of Salicylic acid on photosynthetic content of fenugreek. Each value is mean of 3 replicates and ± values indicate standard errors. Significant difference between treatments is indicated by non-identical letters at p≤0.05. C=Control, NaF= 200ppm NaF, SA1= 5µM SA, SA2=10µM SA, SA3= 15µM SA.

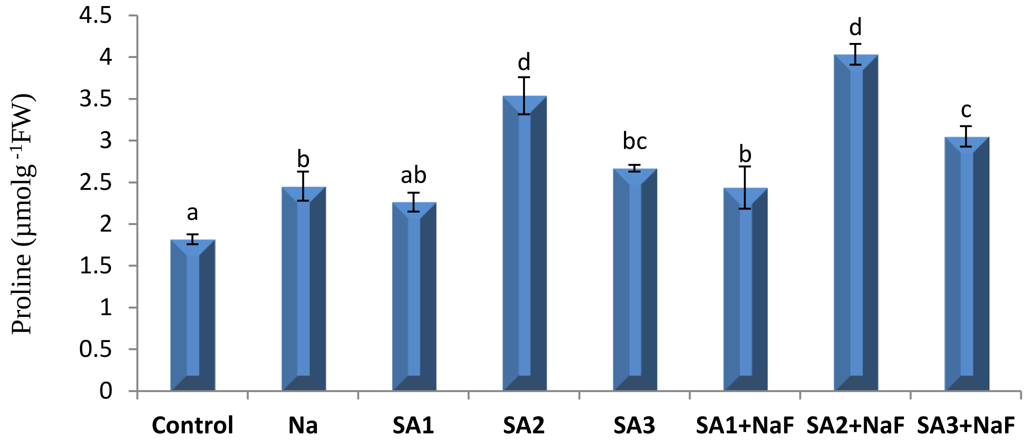
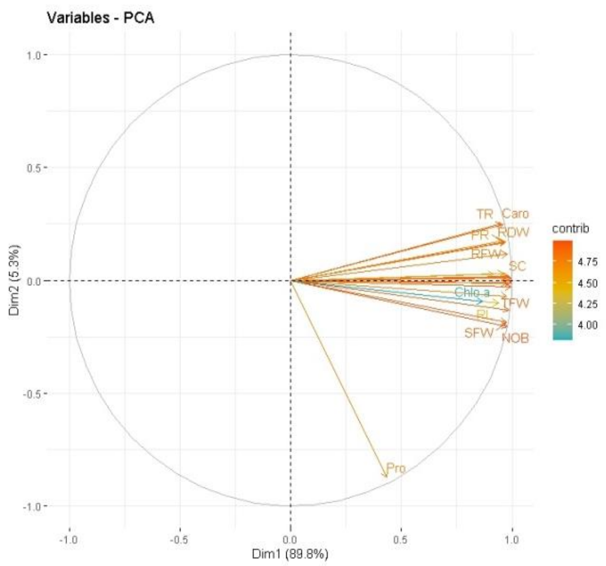


Figure 3: Influence of Salicylic acid on proline content of fenugreek. Each value is mean of 3 replicates and ± values indicate standard errors. Significant difference between treatments is indicated by non-identical letters at p≤0.05. C=Control, NaF= 200ppm NaF, SA1= 5µM SA, SA2=10µM SA, SA3= 15µM SA.



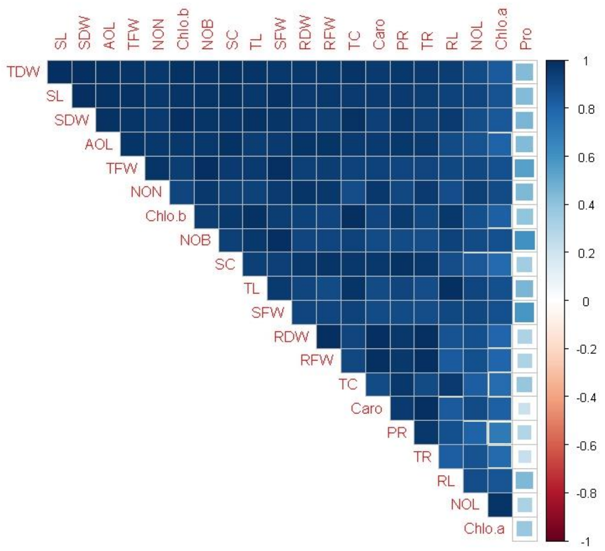


Figure 4: Principal component analysis and Correlation of various traits of fenugreek grown under NaF stress and treated with salicylic acid. The abbreviations given in this figure are follows. TDW (total dry weight), SL (shoot length), SDW (shoot dry weight), AOL (area of leaves), TFW (total fresh weight), NON (number of nodules), Chlo b (chlorophyll *b*), NOB (number of branches) SC (stomatal conductivity), TL (total length), SFW (shoot fresh weight), RDW (root dry weight), RFW (root fresh weight), TC (total Chlorophyll), Caro (carotenoids), PR (photosynthetic rate), TR (transpiration rate), RL (root length), NOL (number of leaves), Chlo a (chlorophyll *a*), pro (proline)

DISCUSSION

Salinity is one of the most significant abiotic factors affecting crop yield and morphological features. Salinity is caused by a variety of natural and artificial sources. Because they lack systems to tolerate salt, the majority of plants are unable to grow on lands that are affected by salinity.[32] Fluoride can enter the human body through food or breathing. At one time, it was believed that humans got little fluoride from food. However, it is now commonly accepted that some dietary products might have high fluoride content. Numerous plants are affected by fluoride poisoning. Fluoride interferes with many metabolic processes, especially those that affect plant growth, chlorophyll content, leaf-tip burn, chlorosis, and necrosis.[33]

To restore both natural and agricultural ecosystems, signaling molecules can reduce the effects of stress on plants and increase output.[34] The plant's antioxidant system reduces stress in plants that are growing under stress. The antioxidant is unable to reduce stress in plants that have experienced significant abiotic stress.[35] Salicylic acid is now widely used as an antioxidant and a plant growth regulator to alleviate salt stress. Salicylic acid contributes to a large number of plant growth and development stages, as well as in defense against various biotic and abiotic stresses.[24]

Fenugreek (*Trigonella foenum-graecum* L.) altered some of its morphological characteristics as a result of sodium fluoride. Compared to the control, the NaF stress significantly decreased the length of the shoot and the root, the area of the leaves, and the concentration of chlorophyll in the fenugreek plant. Increased shoot and root lengths and more resilient seedlings of fenugreek were produced by seeds primed with salicylic acid. The length of the shoots on fenugreek plants under salinity stress improved due to salicylic acid, according to our findings. Significant stress-relieving potential was demonstrated by salicylic acid primed seed with 10 mol L-1 SA concentration (Table 1). A higher rate of cell division and elongation could be the cause of the elongation. The findings produced in this study supported findings that were observed in sweet pepper.[36]

The fresh as well as dry biomass of roots and shoots were significantly reduced due to abiotic stress compared to the control. This decrease in weight might be because of reduction in the metabolic activities of plants. This result is in accordance with the findings of work, in which the reduction in the growth of *Dianthus caryophyllus* was noticed.[37] The plants which were treated with Salicylic acid showed an increasing trend of dry and fresh weight in comparison with those plants in which no Salicylic acid treatment was given. But the increase in biomass was limited to a certain threshold level of Salicylic acid. The increase in biomass was limited to 10 µmol L^{-1} Salicylic acid concentration. At high concentration (15 µmol L^{-1}), a slight decrease in biomass was observed (Table 2). Same kind of results was observed in the findings of Sreelakshmy *et al.* in which the impact of Salicylic acid was observed on the biomass of *Solanum lycopersicum*.[38]

Salinity and Salicylic acid treatment affected root growth more than shoot growth in this investigation, and root responses were quite different than shoot. Previous studies had also described an increase in root biomass as a result of salinity treatment.[39,40] This could be linked to the osmotic adjustment of root cells in return of secondary osmotic stress caused by high salinity. Root allocations of additional photo assimilates are basic plant responses when exposed to the soil or medium salt. The salinity impact was minimized by the utilization of Salicylic acid priming. A less reduction in the growth of root was detected in the case of 10 µmol L^{-1} Salicylic acid (Table 2) which might be because of an increase in absorption area of roots for nutrients as well as for water. These kinds of results were also observed in Idrees *et al.* findings.[41] Increased leaf area and quantity of leaves resulted in higher carbohydrate generation as a result of photosynthesis, resulting in faster development of fenugreek. The number of leaves and leaf area index increases when the concentration of Salicylic acid was applied, but exhibits slight reduction at high concentrations, which is comparable to observations made in pea plants.[42] Parihar *et al.* also found a negative impact on the number of leaves and leaf area of many plants, which were affected by salts.[8]

Salinity highly affected a large number of nodules present on roots of the fenugreek plant. Nitrogen fixation parameters showed high sensitivity to NaF toxicity as formerly revealed in several other legume species like *Medicago sativa*.[43] The main reason for this is nitrogenase activity reduction due to salt concentration. Salicylic acid treatment increased nitrogen-fixing ability of the fenugreek and a large number of root nodules were observed. The current data is in concordance with work done on chickpea[44]. Reduction in the photosynthetic pigment of fenugreek was noted due to Sodium fluoride (Figure 2). The reason might be the oxidation of chlorophyll due to abiotic stress. This stress was mitigated in the treatment in which Salicylic acid was applied. Increased concentrations of chlorophyll *a*, chlorophyll *b*, and carotenoid contents were noted in SA-treated plants. Chlorophyll content as well as carotenoid content of fenugreek plants, that were under salinity stress was improved by Salicylic acid treatment. The current study's observations are accordant with previous research that found salt-induced reductions in photosynthetic pigments can be treated by applying SA.[45] The inhibitory effects on photosynthetic pigments caused by salts were removed and this can be one of the reasons for the stimulation of growth by Salicylic acid under Sodium fluoride stress. These outcomes are in accordance with research on cowpea and alfalfa plants.[23,46]

Sodium fluoride toxicity altered the growth of fenugreek and affected photosynthetic leaf gas exchange parameters like rate of photosynthesis, rate of stomata, and stomatal conductance (Figure1). The reduced rate of photosynthesis might be due to decrease in primary photochemical functions or inhibition of carbon dioxide metabolism. The reduction of stomatal conductance and transpiration rate might be due to decrease in the surface area of leaves or closure of stomata under abiotic stress. This data is in agreement with the work of Christophe *et al.* in which these kinds of results were observed in maize hybrids.[47] Due to Salicylic acid seed priming, augmentation in photosynthetic rate, transpiration rate, and stomatal conductance was analyzed. These results were might be a consequence of the physiological functions of Salicylic acid or less biosynthesis of ethylene. The other reason for the increase in these parameters can be increase in number of leaves and leaf area. These results are in support of previous works done on mung bean and cotton Hamani *et al.* (2020), in which the influence of Salicylic acid on leaf gas exchange parameter of tomato was observed.[48,49]

Salicylic acid at high concentration (15 µmol L-1) showed inhibitory effects. At high concentrations, it negatively affected physiological processes, decreased dry and fresh biomass of fenugreek. Salicylic acid application at the high concentrations not only showed these kinds of impacts on morphological parameters but also on biomass assessment, photosynthetic pigments, and leaf gas exchange parameters. It might be due to decrease in enzyme activities or retardation of growth and photosynthesis. These findings were also depicted in previous works, in which the growth of carrots was checked by applying Salicylic acid as antioxidant.[50] Proline is an organic solute that is involved in osmotic adjustment. Fenugreek accumulates proline as a response to salt stress and water scarcity. Increased proline content in fenugreek was obtained due to NaF toxicity. Gradually accumulation of proline occurred due to abiotic stress. This may be due to reduction in the activities of those enzymes that are involved in degradation of proline. This result is like the previous work which stated increase in proline concentration was observed in sweet pepper in response to salt stress.[14] Significant improvement in proline content was observed due to Salicylic acid (Figure 3). The results of this work are in support of previous works on tomato and sorghum cultivars.[25,51] Sodium fluoride stress resulted in significant reductions in growth indices. This decline in growth traits could be related to decreased absorption of water, disorders in metabolic activity, deficiency of nutrients or the harmful effect caused by excessive salt and chloride ions in the zones of root and then in plant cells. Previously Abd El-Hameid and Sadak also observed these kinds of growth reductions in Sunflower.[2]

Salicylic acid application boosted a number of physical and biological processes, including germination and development of fenugreek. Increase in growth might be due to improvement in many physiological processes such as antioxidant enzymes, proline content as well as photosynthetic pigments due to which mitigation of salt stress happened. The findings of this research can also be related with the work done on wheat crop and bean cultivars, in which increase in growth of wheat was analyzed due to Salicylic acid treatment.[52,53] The results obtained in present study manifested that Salicylic acid reduced salt stress and improved the growth of the plant.

CONCLUSION

The administration of Salicylic acid was used to mitigate Sodium fluoride stress in the current investigation. According to the findings and debate, Salicylic acid is a promising antioxidant that reduces the negative impacts of Sodium fluoride. The application of 200ppm Sodium fluoride by soil drench method resulted in a significant reduction in morphological characteristics as well as biomass when compared with the control. On the other hand, seed priming with Salicylic acid resulted in successful and speedy recovery from NaF stress. Salicylic acid caused a significant rise in plant morphological characteristics and biomass, but only to an optimal level (10 µmol L-1), as Salicylic acid in 15 µmol L^{-1} concentrations showed deleterious impacts on plant growth. Significant improvement in photosynthetic pigments, stomatal conductance, photosynthetic rate and transmission rate occurred with the help of Salicylic acid at 10 µmol L^{-1} concentration. Salinity is constantly worsening and having a negative impact on Pakistani crops, seed priming with antioxidants is a long-term solution for the said problem.

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